A study on chromatography in action biology essay

Science, Biology

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A solution containing as many as 12 components (including n-alkanes, n-alkanols and n-alkanoic acids) were derivatized, separated and analysed using different chromatographic techniques – column chromatography, thin layer chromatography (TLC), gas chromatography (GC) and gas chromatography – mass spectrometry (GC-MS). It was found the techniques in order of effectiveness were as follows CC and TLC followed with GC and GC-MS. Why?

Introduction

Chromatography was first used by Russian botanist Mikhail Tsvet in 1900, to separate plant pigments such as carotene and chlorophyll1. It was further developed from 1940 – 1950 by A. J. P Martin and R. Synge who subsequently won a Nobel prize for their work2. Chromatography refers to a collection of laboratory techniques which are used to separate mixtures. The components in a sample are carried through a stationary phase by a mobile phase at different rates thus causing a separation. There are two different types of chromatography: column chromatography (CC) and planar chromatography (PC). In CC, the stationary phase is held in a tube whilst the mobile phase moves down the tube due to the effect of gravity. PC on the other hand supports the stationary phase on a plate, whilst the mobile phase moves up the plate due to capillary action. Mobile phases are for CC but can be either liquid or gas for PC. 3Column chromatography is a process in which the mixture is washed through a stationary phase by the mobile phase. When a mixture of components enters the column they are separated based

upon their interact with the stationary phase depending on their polarities. Adding fresh eluent (mobile phase) forces the sample through the column allowing elution to occur. 3 In this investigation we will be using a silica gel column Analytes will elute in increasing order of polarity (the most polar component will take the longest travelling through the stationary phase and will require more elute). Thin layer chromatography (TLC) is form of planar chromatography and works by separating mixtures through the movement of a solvent across a flat surface. 4 Plates are most commonly developed by spotting the mixture and reference compounds on a pre-drawn line on the plate near one edge. The plate is then placed in a closed container saturated with vapours of the developing solvent (making sure that there is no direct contact between the sample and the developer). The plate is then removed, the solvent front is marked and the components are then determined in several ways – spraying a solution of iodine or ninhydrin to yield dark products or incorporating a fluorescent material into the stationary phase and the plate can then be examined under an ultra violet light (as the sample components quench the fluorescence of the material, so the sample components can be located in the non-fluorescing sections). 3 In this investigation we will be using DCM as the developing solvent and a solution of Rhodamine B as the indicator. In TLC, the stationary phase is usually a polar solid such as silica gel or alumina. The components of the samples move across the surface of the plate at rates based upon their composition and affinity for the stationary phase. The components are identified by their retardation factors (Rf) which is the ratio of the distance moved by the analyte from the origin to the distance moved by the flowing solvent from

the origin. Each analyte will have and individual Rf value dependent on the conditions. 5 TLC does not however provide quantitative information of high precision or accuracy as one of the main difficulties are defining the boundaries of spots. Relative precision is usually 5 – 10%. 4Gas chromatography (GC) consists of a chemically inert carrier gas (usually Helium or Argon) acting as the mobile phase travelling through a column made up of a liquid phase immobilized onto the surface of an inert sold packing as the stationary phase. Column efficiency requires the sample to be of a suitable size and to be introduced as a plug of vapour to reduce band spreading or poor resolution. A sample injection system can be used to ensure it is of the right size and at the right speed. Once injected it travels through one of two types of columns: packed or capillary. Both are usually made of stainless steel, glass, fused silica of Teflon. These columns are usually formed into coils so that they can fit into an oven for thermosetting. The optimum column temperature depends on the degree of separation required and the boiling point of the sample. 3 Detection for carbon containing compounds is most commonly carried out by flame ionisation detection (FID). Here the effluent is directed into a small hydrogen flame which combusts the sample into fragments, which are then ionized by an electrode. The current produced from these charged ions flowing towards an electrode in the sensor is monitored. 5 Chemical compounds elute from the GC at different rates due to their volatility and interaction with the stationary phase. Interaction between the stationary phase and what? depends on how similar the polarities are. The more similar the polarities, the more interactions and the longer the retention time of the analyte. In regards to

volatility, the more volatile the component, the shorter the retention time. Gas chromatography – mass spectrometry (GC/MS), is the combination of gas chromatography and mass spectrometry. A mass spectrometer measures the mass-to-charge ratio (m/z) of ions that have been produced from the sample. Molecules enter the mass spectrometer through an inlet system after going through the GC. They enter an ionization source which ionizes the sample, leaving them as molecular ions, fragment ions and un-ionized molecules. These are then accelerated under high voltage into a mass analyser which sorts out the ions according to their m/z values. The separated ions are detected by an electron multiplier and a plot of ion intensity versus m/z value is produced by the data system. 3In this investigation, a series of derivatization techniques have been used in order to chemically alter the compounds so that they have more appropriate properties for analysis. Capping functional groups (like alcohols or carboxylic acids) can decrease their polarity and change their volatility. These are favourable properties for chromatographic separations like GC and CC because it allows more control of the retention time. Furthermore, derivatization improves chromatographic resolution and allows for better reproducibility of the analysis. The two derivatization techniques that were implemented in this investigation were esterification (Scheme 1) and silanation (Scheme 2). Scheme 1. Esterification of an alcohol to produce an esterThe carboxylic acids were derivatized with MeOH and Trifluorobornrorh to make a methyl ester. This decreased the polarity of the molecule, which meant that they would pass through the CC faster as there would be less polar interactions with the silica. Scheme 2. Silanation of an alcohol to

produce a silanated alcoholThe alcohols were derivatized with trimethylsilyl chloride in a silylation reaction to produce a silylated alcohol. This – like the esterification – decreased the polarity of the molecule. In this research we aim to investigate which chromatographic techniques are the most useful for separating and analysing a solution containing n-alkanes, n-alkanols and n-alkanoic acids. Once the derivatization has taken place, we would expect the eluting order in CC to go from least polar to most polar – alkanes, esters and then alcohols (due to interactions between the components and the polar stationary phase). We would expect the eluting order in GC to be controlled by the volatility of the molecules and thus their size: the lighter the molecule - the shorter the retention time. Well done it is clear you have done some background research and state your aims.

2. 0 Experimental

The different chromatographic techniques and derivatizations were carried out following the experimental handbook written by the School of Chemistry, University of Bristol. 6All glassware was rinsed with dichloromethane before use.

Preparing Solutions

The dichloromethane solution containing a mixture of components was evaporated on a nitrogen blow-down apparatus and a solution of boron trifluoride and methanol (1 ml, 14% w/w) was added. The vial was capped tightly and heated to 60oC for 30 min. Once the solution had been left to cool, distilled water (1mL) was added. The aqueous solution was extracted with hexane (3 x 2 mL) and the extracts were combined in a 7 mL vial

(labelled A) containing anhydrous magnesium sulphate (0. 1 g). The vial was shaken and left to stand (10 min). Aliquots (2 x 0. 5 mL) of the dried hexane solution were decanted to separate 1 mL vials (labelled B and C). The solvent from vial C was evaporated and N, O-bis(trimethylsilyl)trifuoro-acetamide (BSTFA) containing 1% trimethylsilyl chloride (TMSCL) (4 drops) was added, the vial was capped and it was heated (30 min). Once the solution had been left to cool, hexane (0. 5 ml) was added. This vial was labelled C-TMS.

Column Chromatography (CC)

A slurry of hexane saturated silica gel (3 cm) was poured into a glass chromatography column already containing hexane (ca. 10 cm). The solvent was allowed to flow from the column whilst all the silica was washed down to the bottom. The excess solvent was run out of the column until only 1 cm of solvent remained on top of the silica gel. Solution A (5 mL) was added carefully to the column and the solvent level was adjusted so that it was level with the surface of the silica gel. Hexane (20 mL) was run through the column and collected into a 50 mL round bottomed flask labelled D. This elution was repeated for DCM (20 mL), 1: 1 MeOH/DCM (20 mL) and MeOH (20 mL), with each fraction being collected in a separate RBF. These fractions were evaporated using a rotary evaporator and transferred to 1 ml vials labelled D, E, F and G using DCM (1 mL).

Thin Layer Chromatography (TLC)

A TLC plate (10 cm x 10 cm) was spotted (ca. 15 times) with samples from vials B, D, E, F, G and the following reference compounds: n-hexadecane (1), 1-hexadecanol (2), hexadecanoic acid (3) and methyl hexadecanoate (4).

The plate was developed in a TLC tank containing DCM. Once the solvent front was within 1 cm of the top edge of the plate, the position was marked in pencil. The plate was removed from the tank, allowed to dry (10 min), sprayed with a solution of Rhodamine B, allowed to dry (15 min), viewed under a UV lamp and the positions of the spots were marked with a pencil.

Gas Chromatography (GC)

Fraction F was evaporated to dryness and N, O-bis(trimethylsilyl)trifuoro-acetamide (BSTFA) containing 1% trimethylsilyl chloride (TMSCL) (4 drops) was added, the vial was capped and it was heated (30 mins). This was now labelled F-deriv. Separate GC autosampler vials were half filled with each of the samples C-TMS, D, E and F-deriv. GC analyses were performed on a GC2014. Samples were injected via an injection port SPL1 into a fused silica capillary column (30m x 0. 25mm). The temperature programme consisted of a 1 min isothermal period at 50oC followed by an increase to 300oC at 10oC min-1. Held? He was the carrier gas and a flame ionisation detector was used to detect the column eluent. Brand of instrument? Flow rate? Column name?

Gas Chromatography – Mass Spectrometry (GC-MS)

A GC/MS vial was half filled with C-TMS. Analyses of the C-TMS sample were performed on a GC-2010 interfaced to a quadrupole 2010 MS (scan range m/z 50-650; scan time 0. 6 sec). The column and temperature program used were the same as those for GC analyses with He as the carrier gas. The total ion chromatogram (TIC) was recorded along with the mass spectra of the various components seen in the chromatogram and three characteristic

mass chromatograms were plotted which allowed us to selectively reveal each of the three compound classes present in the mixture. Ionisation method? Generally well written.

3. 0 Results and discussion

Column Chromatography (CC) and Thin Layer Chromatography (TLC)

Figure 1. A visual representation of a TLC plate of n-hexadecane (1), 1-hexadecanol (2), hexadecanoic acid (3) and methyl hexadecanoate (4) and of a set of fractions, D, E, F, G and B separated by CC.

Sample

Rf Value

10. 8220. 23

-

40. 62D0. 78E0. 60F0. 2G

-

B0. 19, 0. 64, 0. 75Table 1. Rf values from the TLC plate of n-hexadecane (1), 1-hexadecanol (2), hexadecanoic acid (3) and methyl hexadecanoate (4) and of a set of fractions, D, E, F, G and B separated by CC. Results show the following samples have the same Rf values (Table 1): D and hexadecane (1), E and methyl hexadecanoate (4) and F and 1-hexadecanol (2). Hexadecanoic acid (3) had too much spreading to be identified, G had no spots on the plate and B had matching Rf values with 1, 2 and 4. Sample G did not appear on the TLC plate (Figure 1) because there were no components present in it. All

the fractions had already run off the column in previous eluates and thus when it came to running MeOH through the column, no components were left in the column to dissolve into the mobile phase. Samples D, E and F all had matching Rf values with specific reference spots – thus implying their functional group presence in the individual fractions: Alkanes in D, Esters in E and Alcohols in F. Sample B has three matching Rf values with the samples and fractions because it was a sample of the mixture before being run through the column. Therefore all three components would be expected to be in this. Can discuss further i. e. why do n-alkanes have a higher Rf than the alcohols?

Gas Chromatography (GC)

Fraction F was evaporated and derivatised using N, O-bis(trimethylsilyl)trifuoro-acetamide (BSTFA) containing 1% trimethylsilyl chloride (TMSCL). This was heated and put into a GC vial. C-TMS, D, E and G were also put into separate GC vials and the retention times of the major components eluting after 10 min was recorded. Figure 2. Gas Chromatogram of C-TMS and samples D, E, F, and G

Peak number

Samples

Retention times (min)

C-TMSDEFG113. 80413. 804214. 80914. 810315. 19615. 204417. 91617. 925518. 00218. 002618. 74818. 747721. 49221. 502821. 58021. 583922. 11522. 145Table 2. GC retention times of samples C-TMS, D, E, F and GThe data from the gas chromatogram from the four samples (Table 2) – D, E, F

and G - shows that there are three peaksin each which match retention times of the peaks in C-TMS. There are trace amounts of other compounds in E and F (Figure 2) which was probably due to the separation abilities of CC. No compounds were present in sample G confirming conclusions drawnfrom the TLC. Table is a nice way of showing your data. Now that you know which compound classes elute in which fraction you could add this.

Gas Chromatography – Mass Spectrometry (GC/MS) definitely not spectroscopy – do you know why?

A GC-MS was run on C-TMS and the total ion chromatogram (TIC) was recorded along with the mass spectra of the various components seen in the chromatogram. Three characteristic mass chromatograms were plotted which allowed us to identify each of the three compound classes present in the mixture. Figure 3. Total Ion Chromatogram of C-TMS

Would be useful if you could label your peaks here, and include label axes.

Figure 4. Mass spectrum of component peaks 1-9You could annotate these spectra and discuss diagnostic ions in further detail. Figure 5. Three characteristic chromatograms of three fragments A, B and CIt is known that the mixture that is being separated contains alkanes, derivatized alcohols and derivatized esters. Therefore through reasoning we can match up fragment ions to the characteristic m/z values obtained.

Chromatogram

m/z

Fragment Ion

A57B74McClafferty ion? C103Table 3. Characteristic fragment ions found in the mass spectrum (refer to Figure 4 and 5)

Peak number

Characteristic Fragment

Functional Group

Molecular Name

174EsterMethyl Deconate2103Silanated AlcoholTrimethylsiloxydecane357AlkaneTetradecane457AlkaneHexadecane574EsterTridecanoic acid6103Silanated AlcoholNo similarity match757AlkaneNonadecane874EsterHexadecanoic acid9103Silanated AlcoholTrimethylsiloxyhexadecaneTable 4:- Identified peaks from the total ion chromatogram. The data from the GC-MS allows us to identify the components from the GC. Each GC instrument will give different retention times of a mixture – however the order that the mixture elutes out of the instrument does not change. Therefore we can transpose the order of peaks from the GC onto the GC-MS.

Sample

Molecules Present

DTetradecaneHexadecaneNonadecaneEMethyl DecanoateTridecanoic AcidHexadecanoic AcidFTrimethylsiloxydecaneAn unknown silanated

alcoholTrimethylsiloxyhexadecaneTable 5:- Identified molecules present in original fractions from CCFrom TLC analysis we expect sample D to contain alkanes, E to contain esters and F to contain silanated alcohols. Taking the information from the GC-MS we can see that this information is echoed (Table 5). However, two of the molecules present in sample E have been identified as carboxylic acids instead of esters – implying that derivatization was not that successful. Your discussion section is very brief. You should refer to each of your figures in the text. You are lacking a discussion of the mass spectra themselves- what fragments mean, which is the molecular ion etc. Well done for including mass chromatograms.

5. 0 Conclusions

CC successfully separated the mixture into three different fractions. TLC verified this separation by comparing it with reference samples. GC managed to further separate these fractions into nine different components which could then be identified using GC-MS. This sequence of events highlights the effectiveness of separation and identification of the different techniques. Separating with CC and identifying with TLC only allowed for tentative identifications about what was present in the mixture (we could only conclude the mixture was made up of three different functional groups). Separating with GC and identifying with GC-MS on the other hand allowed us to make more specific conclusions about the molecules present in the mixture – including their actual identity. Good, but you should have discussed more of the mechanisms underlying the separation techniques and made comparisons to the literature. If I were to repeat this experiment, I

would make sure that the molecules were correctly derivatised so that sample E would contain only esters (and not a mixture of carboxylic acids too) and then I would hopefully be able to identify all of the silanated alcohols present.

Acknowledgments

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Feedback Reflection

In previous reports I have been given feedback in regards to including more background of the topic in my introduction, keeping my referencing style constant, including more analysis when discussing results found from spectroscopic techniques and to improve the links between the text and diagrams by using the correct scientific method. I have tried to encompass all of these ideas into this report, hopefully resulting in a coherent piece of writing. Your report is well-written and there is good treatment of the data in terms of tables and figures, but remember to refer to them in the text and to label your axes. You should expand your discussion – particularly of the MS data.